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I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation and Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General hereby certify that annexed hereto is a true copy of the international application filed on 11 September 1997 under the Patent Cooperation Treaty at the UK Receiving Office. The application was allocated the number PCT/GB97/02478.

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Signed T A Roberts.

Dated 26 JANUARY 2001

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REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

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PCT/GB 97 / 02478
International Application No.

11 SEPTEMBER 1997 / 11 09 97
International Filing Date

United Kingdom Patent Office
PCT International Application
Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired)(12 characters maximum)* REP05291WO

Box No. I TITLE OF INVENTION
NUCLEIC ACID PARTICLE DELIVERY

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

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This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANTS AND/OR (FURTHER) INVENTORS

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Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

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PCT/GB 97 / 02478

Continuation of Box No. III FURTHER APPLICANTS AND/OR (FURTHER) INVENTORS

If none of the following sub-boxes is used, this sheet is not to be included in the request.

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This person is applicant for the purposes of:
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- [X] applicant and inventor
- [ ] inventor only *(if this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality: AU

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This person is applicant for the purposes of:
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Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(if this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality:

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Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(if this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality:

State *(i.e. country)* of residence:

This person is applicant for the purposes of:
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The following designations are hereby made under Rule 4.9(a) (*mark the applicable check-boxes; at least one must be marked*):

Regional Patent

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PCT/GB 97/02478

Box No. VI PRIORITY CLAIM Further priority claims are indicated in the Supplemental Box ☐

The priority of the following earlier applications is hereby claimed:

Country *(in which, or for which, the application was filed)*	Filing Date *(day/month/year)*	Application No.	Office of filing *(only for regional or international application)*
item (1) GB	11 September 1996 11.09.96	9619002.0	
item (2)			
item (3)			

Mark the following check-box if the certified copy of the earlier application is to be issued by the Office which for the purposes of the present international application is the receiving Office (a fee may be required):

☒ The receiving Office is hereby requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) identified above as item(s) : (1)

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):* ISA/_____

Earlier Search *Fill in where a search (international, international-type or other) by the International Searching Authority has already been carried out or requested and the Authority is now requested to base the international search, to the extent possible, on the results of that earlier search. Identify such search or request either by reference to the relevant application (or the translation thereof) or by reference to the search request:*

Country (or regional Office): Date *(day/month/year)*: Number:

Box No. VIII CHECK LIST

This international application contains the following number of sheets:

1. request	4	sheets
2. description	34	sheets
3. claims	2	sheets
4. abstract	1	sheets
5. drawings	2	sheets
Total	43	sheets

This international application is accompanied by the item(s) marked below:

1. ☐ separate signed power of attorney
2. ☐ copy of general power of attorney
3. ☐ statement explaining lack of signature
4. ☐ priority document(s) *identified in Box No. VI as item(s):*
5. ☒ fee calculation sheet
6. ☐ separate indications concerning deposited microorganisms
7. ☐ nucleotide and/or amino acid sequence listing (diskette)
8. ☒ other *(specify)*: Patents Form 23/77

Figure No. _____ of the drawings (if any) should accompany the abstract when it is published.

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

For the Applicant
Gill Jennings & Every

PERRY, Robert Edward

Date: 11 September 1997

For receiving Office use only

1. Date of actual receipt of the purported international application: 11 SEPTEMBER 1997 / 11 09 97

2. Drawings: ☑ received: ☐ not received:

3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:

4. Date of timely receipt of the required corrections under PCT Article 11(2):

5. International Searching Authority specified by the applicant: ISA/

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NUCLEIC ACID PARTICLE DELIVERY

Technical Field

The present invention relates generally to DNA delivery methods. More particularly, the invention pertains to *in vivo* and *ex vivo* delivery of powdered nucleic acid molecules into mammalian tissue using needleless injection techniques.

Background of the Invention

Gene therapy and DNA immunization are promising approaches for the treatment and prevention of both acquired and inherited diseases. These techniques provide for the transfer of a desired gene into a subject with the subsequent *in vivo* expression thereof. Gene transfer can be accomplished by transfecting the subject's cells or tissues *ex vivo* and reintroducing the transformed material into the host. Alternatively, genes can be administered directly to the recipient.

A number of methods have been developed for gene delivery in these contexts. For example, viral-based systems using, e.g., retrovirus, adenovirus, and adeno-associated viral vectors, have been developed for gene delivery. However, these systems pose the risk of delivery of replication-competent viruses. Hence, nonviral methods for direct transfer of genes into recipient cells and tissues are desirable.

Nonviral methods of gene transfer often rely on mechanisms employed by mammalian cells for the uptake and intracellular transport of macromolecules. For example, receptor-mediated methods of gene transfer have been

developed. The technique utilizes complexes between plasmid DNA and polypeptide ligands that can be recognized by cell surface receptors. However, data suggests that this method may permit only transient expression of genes and thus has only limited application.

Additionally, microinjection techniques have been developed for the direct injection of genetic material into cells. The technique, however, is laborious and requires single cell manipulations. Thus, the method is inappropriate for use on a large scale.

Direct injection of DNA-containing solutions into the interstitial space for subsequent uptake by cells has also been described. For example, International Publication No. WO 90/11092, published 4 October 1990, describes the delivery of isolated polynucleotides to the interior of cells wherein the isolated polynucleotides are delivered into the interstitial space of the tissue and then taken up by individual cells to provide a therapeutic effect. Such methods entail the injection of the DNA-containing solutions into tissue using conventional needles or cannulas, and are therefore not well suited for long term therapies or for field or home applications.

Biolistic particle delivery systems (particle bombardment systems) have also been developed for gene delivery into plant cells. Such techniques use a "gene gun" to introduce DNA-coated microparticles, such as DNA-coated metals, into cells at high velocities. The coated metals are generally propelled into cells using an explosive burst of an inert gas such as helium. *See, e.g.*, U.S. Patent No. 5,100,792 to Sanford et al. The technique allows for the direct, intracellular delivery of small amounts of DNA.

Tungsten or gold particle microprojectiles are generally needed to achieve adequate gene transfer frequency by such direct injection techniques. In particular, these materials have been selected based on their availability in defined particle sizes around 1 μm in diameter, as well as having a sufficiently high density to achieve the momentum required for cell wall penetration. Additionally, the metals used are chemically inert to reduce the likelihood of explosive oxidation of fine microprojectile powders, as well as non-reactive with DNA and other components of the precipitating mixes, and display low toxicity to target cells. See e.g., *Particle Bombardment Technology for Gene Transfer*, (1994) Yang, N. ed., Oxford University Press, New York, NY pages 10-11.

However, there is evidence that tungsten toxicity can reduce the recovery of stable transformants. Additionally, such biolistic techniques are not appropriate for use with large DNA molecules since precipitation of such molecules onto metal carriers can lead to unstable configurations which will not withstand the shear forces of gene gun delivery. Furthermore, metal carriers are retained by tissues and/or cells and may cause discoloration, as well as a number of other undesirable biological effects particularly in the case of direct delivery to internal tissues, or direct treatment of cells using *ex vivo* techniques. Thus, the use of a gene gun to deliver DNA-coated metal particles is likely to be problematic for repeated therapy, especially in mammalian subjects.

Accordingly, there remains a need to provide a highly efficient method for introducing therapeutically relevant DNA or other nucleic acid molecules into mammalian tissue cells wherein the method avoids the

problems commonly encountered with prior gene delivery techniques.

Disclosure of the Invention

The present invention is based on the surprising discovery that solid particles of nucleic acid molecules having a nominal average diameter of at least about 10 μm and which are therefore larger than the average mammalian cell, can be delivered into the cells of mammalian tissue for highly efficient gene transfer. The result is unexpected because it was heretofore believed that only small DNA-coated metallic particles, having a much smaller size than typical mammalian cells, could adequately be used as microprojectiles in biolistic gene delivery techniques. See e.g., *Particle Bombardment Technology for Gene Transfer*, (1994) Yang, N. ed., Oxford University Press, New York, NY pages 10-11.

In the practice of the invention, powdered nucleic acid molecules are delivered using needleless injection techniques. In particular, a novel delivery system that uses a needleless syringe to fire solid particles of therapeutic agents in controlled doses into and through intact skin has recently been described in commonly assigned International Publication No. WO 94/24263, published 27 October 1994. The publication describes a needleless syringe that delivers pharmaceutical particles entrained in a supersonic gas flow. The needleless syringe can be used for transdermal delivery of powdered drug compounds and compositions, for delivery of genetic material into living cells (e.g., gene therapy) and for the delivery of biopharmaceuticals to skin, muscle, blood or lymph. The needleless syringe can also be used in conjunction with surgery to deliver drugs and biologics to organ surfaces, solid tumors

and/or to surgical cavities (e.g., tumor beds or cavities after tumor resection).

Accordingly, in one embodiment, the invention is directed to a method for delivering solid particles comprised of nucleic acid molecules to mammalian tissue for the genetic transformation of cells in the tissue with the delivered nucleic acids. In a substantial departure from conventional particle bombardment techniques, the nucleic acid particles transferred using the method of the present invention are not delivered using dense metal carriers. Furthermore, the molecules have a particle size that is equal to or larger than the average mammalian cell size.

More particularly, densified particles comprised of selected nucleic acid molecules and, optionally, suitable carriers or excipients, are prepared for delivery to mammalian tissue via a needleless syringe which is capable of expelling the particles at supersonic delivery velocities of between Mach 1 and Mach 8. The particles have an average size that is at least about 10 μm, wherein an optimal particle size is usually at least about 10 to 15 μm (equal to or larger than the size of a typical mammalian cell). However, nucleic acid particles having average particle sizes of 250 μm or greater can also be delivered using the present method. The depth that the delivered particles will penetrate the targeted tissue depends upon particle size (e.g., the nominal particle diameter assuming a roughly spherical particle geometry), particle density, the initial velocity at which the particle impacts the tissue surface, and the density and kinematic viscosity of the tissue. In this regard, optimal individual particle densities (e.g., in contrast to bulk powder density) for use in needleless injection generally range between about

0.1 and 25 g/cm^3, and injection velocities generally range between about 200 and 3,000 m/sec.

In various aspects of the invention, the above method can be practiced *in vivo* to provide targeted delivery of the nucleic acid particles, such as delivery to the epidermis (for gene therapy applications) or to the *stratum basal* layer of skin (for nucleic acid immunization applications). In these aspects of the invention, particle characteristics and/or device operating parameters are selected to provide tissue-specific delivery. One particular approach entails the selection of particle size, particle density and initial velocity to provide a momentum density (e.g., particle momentum divided by particle frontal area) of between about 2 and 10 kg/sec/m, and more preferably between about 4 and 7 kg/sec/m. Such control over momentum density allows for precisely controlled, tissue-selective delivery of the nucleic acid particles.

In other aspects of the invention, the needleless syringe is used to transfect cells or tissues *ex vivo* with the particulate nucleic acid molecules, wherein the transformed cells are subsequently reintroduced into the host.

These and other embodiments of the subject invention will readily occur to those of skill in the art in light of the disclosure herein.

Brief Description of the Figures

Figure 1 is a pictorial representation of an *ex vivo* delivery apparatus having a needleless syringe arranged over a tissue culture plate containing cells to be transformed with the particulate nucleic acid preparations described herein.

Figure 2 is a histogram depicting transformation efficiencies obtained using the apparatus

of Figure 1 to deliver DNA particles at 30 bar pressure over a 60 mm target distance as described in the Example.

Figure 3 is a graph depicting the transformation efficiencies obtained using the apparatus of Figure 1 to deliver DNA particles at 30 bar pressure over a range of target distances, also as described in the Example.

Detailed Description of the Preferred Embodiments

The practice of the present invention will employ, unless otherwise indicated, conventional methods of molecular biology and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Sambrook, et al., *Molecular Cloning: A Laboratory Manual* (2nd Edition, 1989); Maniatis et al., *Molecular Cloning: A Laboratory Manual* (1982); DNA Cloning: A Practical Approach, vol. I & II (D. Glover, ed.); Perbal, A Practical Guide to Molecular Cloning.

It must be noted that, as used in this specification and the appended claims, the singular forms "a", "an" and "the" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to "a nucleic acid molecule" includes a mixture of two or more nucleic acid molecules, reference to "an excipient" includes mixtures of two or more excipients, and the like.

A. Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. The following terms are intended to be defined as indicated below.

"Gene delivery" refers to methods or systems for reliably inserting foreign DNA into host cells. Such methods can result in expression of non-integrated transferred DNA, extrachromosomal replication and expression of transferred replicons (e.g., episomes), or integration of transferred genetic material into the genomic DNA of host cells.

The nucleotide sequences are generally present in a suitable nucleic acid molecule and delivered in the form of vectors. By "vector" is meant any genetic element, such as a plasmid, phage, transposon, cosmid, chromosome, virus, virion, etc., which is capable of replication when associated with the proper control elements and which can transfer gene sequences between cells.

A "nucleotide sequence" or a "nucleic acid molecule" refers to DNA and RNA sequences. The term captures molecules that include any of the known base analogues of DNA and RNA such as, but not limited to 4-acetylcytosine, 8-hydroxy-N6-methyladenosine, aziridinylcytosine, pseudoisocytosine, 5-(carboxyhydroxylmethyl) uracil, 5-fluorouracil, 5-bromouracil, 5-carboxymethylaminomethyl-2-thiouracil, 5-carboxymethylaminomethyluracil, dihydrouracil, inosine, N6-isopentenyladenine, 1-methyladenine, 1-methylpseudouracil, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-methyladenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarbonylmethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid, oxybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil,

N-uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid, pseudouracil, queosine, 2-thiocytosine, and 2,6-diaminopurine.

A "coding sequence" or a sequence which "encodes" a particular polypeptide, is a nucleic acid sequence which is transcribed (in the case of DNA) and translated (in the case of mRNA) into a polypeptide *in vitro* or *in vivo* when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are conventionally determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxy) terminus. A coding sequence can include, but is not limited to, cDNA from procaryotic or eukaryotic mRNA, genomic DNA sequences from procaryotic or eukaryotic DNA, and even synthetic DNA sequences. A transcription termination sequence will usually be located 3' to the coding sequence.

The term DNA "control sequences" refers collectively to promoter sequences, polyadenylation signals, transcription termination sequences, upstream regulatory domains, origins of replication, internal ribosome entry sites ("IRES"), enhancers, and the like, which collectively provide for the replication, transcription and translation of a coding sequence in a recipient cell. Not all of these control sequences need always be present so long as the selected gene is capable of being replicated, transcribed and translated in an appropriate recipient cell.

"Operably linked" refers to an arrangement of elements wherein the components so described are configured so as to perform their usual function. Thus, control sequences operably linked to a coding sequence are capable of effecting the expression of the coding sequence. The control sequences need not be contiguous with the coding sequence, so long as they function to

direct the expression thereof. Thus, for example, intervening untranslated yet transcribed sequences can be present between a promoter sequence and the coding sequence and the promoter sequence can still be considered "operably linked" to the coding sequence.

By "isolated" when referring to a nucleotide sequence, or a nucleic acid molecule containing the nucleotide sequence, is meant that the indicated molecule is present in the substantial absence of other biological macromolecules of the same type. Thus, an "isolated nucleic acid molecule which encodes a particular polypeptide" refers to a nucleic acid molecule which is substantially free of other nucleic acid molecules that do not encode the subject polypeptide; however, the molecule may include some additional bases or moieties which do not deleteriously affect the basic characteristics of the composition.

The term "transfection" is used to refer to the uptake of foreign DNA by a host cell, and a host cell has been "transformed" as a result of having been transfected. The foreign DNA may or may not be integrated (covalently linked) to chromosomal DNA making up the genome of the cell. By "host cell," or "host mammalian cell" is meant a cell which has been transfected, or is capable of being transfected, by a nucleic acid molecule containing a nucleotide sequence of interest. The term includes the progeny of the parent cell, whether or not the progeny is identical in morphology or in genetic make-up to the original parent, so long as the nucleotide sequence of interest is present within the cell.

The term "transdermal" delivery captures both transdermal (or "percutaneous") and transmucosal administration, i.e., delivery by passage of a drug or pharmaceutical agent through the skin or mucosal tissue.

See, e.g., *Transdermal Drug Delivery: Developmental Issues and Research Initiatives*, Hadgraft and Guy (eds.), Marcel Dekker, Inc., (1989); *Controlled Drug Delivery: Fundamentals and Applications*, Robinson and Lee (eds.), Marcel Dekker Inc., (1987); and *Transdermal Delivery of Drugs*, Vols. 1-3, Kydonieus and Berner (eds.), CRC Press, (1987). Aspects of the invention which are described herein in the context of "transdermal" delivery, unless otherwise specified, are meant to apply to both transdermal and transmucosal delivery. That is, the compositions, systems, and methods of the invention, unless explicitly stated otherwise, should be presumed to be equally applicable to transdermal and transmucosal modes of delivery.

The above nucleic acid molecules, alone or in combination with drugs or other therapeutic agents, are typically prepared as particulate compositions which generally contain one or more added materials such as carriers, vehicles, and/or excipients. "Carriers," "vehicles" and "excipients" are normally substantially inert materials which are nontoxic and do not interact with other components of the composition in a deleterious manner. These materials can be used to increase the amount of solids in particulate pharmaceutical compositions, such as those prepared using spray-drying or lyophilization techniques, as described further below. Examples of suitable carriers include silicone, gelatin, waxes, and like materials. Examples of normally employed "excipients" or "carriers" include pharmaceutical grades of dextrose, sucrose, lactose, trehalose, mannitol, sorbitol, inositol, dextran, starch, cellulose, sodium or calcium phosphates, calcium sulfate, citric or tartaric acids (and pharmaceutically acceptable salts thereof), glycine, high molecular weight polyethylene glycols (PEG), and combinations thereof. Exemplary excipients

that serve as stabilizers include commonly available cryoprotectants and antioxidants.

B. Modes of Carrying Out the Invention

Before describing the present invention in detail, it is to be understood that this invention is not limited to particular formulations or process parameters as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments of the invention only, and is not intended to be limiting.

Although a number of methods and materials similar or equivalent to those described herein can be used in the practice of the present invention, the preferred materials and methods are described herein.

As explained above, the present invention allows for the highly efficient delivery of solid particles of nucleic acid molecules having a nominal average diameter of at least about 10 μm, to mammalian tissues and cells. The method utilizes biolistic gene transfer techniques yet allows for the delivery of nucleic acid molecules without the need for metal carriers.

A wide variety of nucleic acid molecules can be delivered using the methods of the invention. Generally, the molecules contain coding regions with suitable control sequences or other therapeutically relevant nucleotide sequences. The nucleic acid molecules are prepared in the form of vectors which include the necessary elements to direct transcription and translation in a host cell. If expression is desired using the host's enzymes (such as by the use of endogenous RNA polymerase), the gene or genes will be present in the vectors operatively linked to control sequences recognized by the particular host, or even

particular cells within the host. Thus, eucaryotic and phage control elements will be present for expression in mammalian hosts. Such sequences are known in the art and are discussed more fully below.

Suitable nucleotide sequences for use in the delivery methods of the present invention include any therapeutically relevant nucleotide sequence. Thus, the present invention can be used to deliver one or more genes encoding a protein defective or missing from a target cell genome or one or more genes that encode a non-native protein having a desired biological or therapeutic effect (e.g., an antiviral function). The invention can also be used to deliver a nucleotide sequence capable of providing immunity, for example an immunogenic sequence that serves to elicit a humoral and/or cellular response in a subject, or a sequence that corresponds to a molecule having an antisense or ribozyme function.

Suitable genes which can be delivered include those used for the treatment of inflammatory diseases, autoimmune, chronic and infectious diseases, including such disorders as AIDS, cancer, neurological diseases, cardiovascular disease, hypercholestemia; various blood disorders including various anemias, thalassemia and hemophilia; genetic defects such as cystic fibrosis, Gaucher's Disease, adenosine deaminase (ADA) deficiency, emphysema, etc. A number of antisense oligonucleotides (e.g., short oligonucleotides complementary to sequences around the translational initiation site (AUG codon) of an mRNA) that are useful in antisense therapy for cancer and for viral diseases have been described in the art. See, e.g., Han et al. (1991) *Proc. Natl. Acad. Sci. USA* 88:4313; Uhlmann et al. (1990) *Chem. Rev.* 90:543; Helene et al. (1990) *Biochim. Biophys. Acta.* 1049:99; Agarwal et al. (1988) *Proc. Natl. Acad. Sci. USA* 85:7079; and

Heikkila et al. (1987) *Nature* 328:445. A number of ribozymes suitable for use herein have also been described. See, e.g., Cech et al. (1992) *J. Biol. Chem.* 267:17479 and U.S. Patent No. 5,225,347 to Goldberg et al.

For example, in methods for the treatment of solid tumors, genes encoding toxic peptides (i.e. chemotherapeutic agents such as ricin, diphtheria toxin and cobra venom factor), tumor suppressor genes such as p53, genes coding for mRNA sequences which are antisense to transforming oncogenes, antineoplastic peptides such as tumor necrosis factor (TNF) and other cytokines, or transdominant negative mutants of transforming oncogenes, can be delivered for expression at or near the tumor site.

Similarly, genes coding for peptides known to display antiviral and/or antibacterial activity, or stimulate the host's immune system, can also be administered. Thus, genes encoding many of the various cytokines (or functional fragments thereof), such as the interleukins, interferons, and colony stimulating factors, will find use with the instant invention. The gene sequences for a number of these substances are known.

For the treatment of genetic disorders, functional genes corresponding to genes known to be deficient in the particular disorder can be administered to the subject. The instant methods will also find use in antisense therapy, e.g., for the delivery of oligonucleotides able to hybridize to specific complementary sequences thereby inhibiting the transcription and/or translation of these sequences. Thus, DNA or RNA coding for proteins necessary for the progress of a particular disease can be targeted, thereby disrupting the disease process. Antisense therapy, and

numerous oligonucleotides which are capable of binding specifically and predictably to certain nucleic acid target sequences in order to inhibit or modulate the expression of disease-causing genes are known and readily available to the skilled practitioner. Uhlmann et al. (1990) *Chem. Rev.* 90:543, Neckers et al (1992) *Crit. Rev. Oncogenesis* 3:175; Simons et al. (1992) *Nature* 359:67; Bayever et al. (1992) *Antisense Res. Dev.* 2:109; Whitesell et al. (1991) *Antisense Res. Dev.* 1:343; Cook et al. (1991) *Anti-Cancer Drug Design* 6:585; Eguchi et al. (1991) *Annu. Rev. Biochem.* 60:631. Accordingly, antisense oligonucleotides capable of selectively binding to target sequences in host cells are provided herein for use in antisense therapeutics.

For nucleic acid immunizations, antigen-encoding expression vectors can be delivered to a subject for the purpose of eliciting humoral and/or cellular immune responses to antigens encoded by the vector. In particular, humoral, cytotoxic cellular and protective immune responses elicited by direct intramuscular injection of antigen-encoding DNAs have been described. Tang et al. (1992) *Nature* 358:152; Davis et al. (1993) Hum. Molec. Genet. 2:1847; Ulmer et al. (1993) *Science* 258:1745; Wang et al. (1993) *Proc. Natl. Acad. Sci. USA* 90:4156; Eisenbraun et al. (1993) *DNA Cell Biol.* 12:791; Fynan et al. (1993) *Proc. Natl. Acad. Sci. USA* 90:12476; Fuller et al. (1994) *AIDS Res. Human Retrovir.* 10:1433; and Raz et al. (1994) *Proc. Natl. Acad. Sci. USA* 91:9519.

Modes of carrying out the invention are described more fully below.

Isolation of Genes and Construction of Vectors:

Nucleotide sequences selected for use in the present invention can be derived from known sources, for example, by isolating the same from cells containing a

desired gene or nucleotide sequence using standard techniques. Similarly, the nucleotide sequences can be generated synthetically using standard modes of polynucleotide synthesis that are well known in the art. See, e.g., Edge et al. (1981) *Nature* 292:756; Nambair et al. (1984) *Science* 223:1299; Jay et al. (1984) *J. Biol. Chem.* 259:6311. Generally, synthetic oligonucleotides can be prepared by either the phosphotriester method as described by Edge et al. (*supra*) and Duckworth et al. (1981) *Nucleic Acids Res.* 9:1691, or the phosphoramidite method as described by Beaucage et al. (1981) *Tet. Letts.* 22:1859, and Matteucci et al. (1981) *J. Am. Chem. Soc.* 103:3185. Synthetic oligonucleotides can also be prepared using commercially available automated oligonucleotide synthesizers. The nucleotide sequences can thus be designed with appropriate codons for a particular amino acid sequence. In general, one will select preferred codons for expression in the intended host. The complete sequence is assembled from overlapping oligonucleotides prepared by standard methods and assembled into a complete coding sequence. *See, e.g.*, Edge et al. (*supra*); Nambair et al. (*supra*) and Jay et al. (*supra*).

A particularly convenient method for obtaining nucleic acid sequences for use herein is by recombinant means. Thus, a desired nucleotide sequence can be excised from a plasmid carrying the same using standard restriction enzymes and procedures. Site specific DNA cleavage is performed by treating with the suitable restriction enzyme (or enzymes) under conditions which are generally understood in the art, and the particulars of which are specified by manufacturers of commercially available restriction enzymes. If desired, size separation of the cleaved fragments may be performed by

polyacrylamide gel or agarose gel electrophoresis using standard techniques.

Restriction cleaved fragments may be blunt ended by treating with the large fragment of *E. coli* DNA polymerase I (Klenow) in the presence of the four deoxynucleotide triphosphates (dNTPs) using standard techniques. The Klenow fragment fills in at 5' single-stranded overhangs but digests protruding 3' single strands, even though the four dNTPs are present. If desired, selective repair can be performed by supplying only one, or several, selected dNTPs within the limitations dictated by the nature of the overhang. After Klenow treatment, the mixture can be extracted with e.g. phenol/chloroform, and ethanol precipitated. Treatment under appropriate conditions with S1 nuclease or BAL-31 results in hydrolysis of any single-stranded portion.

Once coding sequences for desired proteins have been prepared or isolated, such sequences can be cloned into any suitable vector or replicon. Numerous cloning vectors are known to those of skill in the art, and the selection of an appropriate cloning vector is a matter of choice. Ligations to other sequences are performed using standard procedures, known in the art.

Selected nucleotide sequences can be placed under the control of regulatory sequences such as a promoter, ribosome binding site and, optionally, an operator (collectively referred to herein as "control" elements), so that the sequence encoding the desired protein is transcribed into RNA in the host tissue transformed by a vector containing this expression construct. The coding sequence may or may not contain a signal peptide or leader sequence.

The choice of control elements will depend on the host being transformed and the type of preparation

used. Thus, if the host's endogenous transcription and translation machinery will be used to express the proteins, control elements compatible with the particular host will be utilized. In this regard, several promoters for use in mammalian systems are known in the art and include, but are not limited to, promoters derived from SV40, CMV, HSV, RSV, MMTV, T7, T3, among others. Similarly, promoters useful with procaryotic enzymes are known and include the tac, spa, trp, trp-lac λ-p_L, T7, phoA promoters, as well as others.

In addition to control sequences, it may be desirable to add regulatory sequences which allow for regulation of the expression of protein sequences encoded by the delivered nucleotide sequences. Regulatory sequences are known to those of skill in the art, and examples include those which cause the expression of a coding sequence to be turned on or off in response to a chemical or physical stimulus, including the presence of a regulatory compound. Other types of regulatory elements may also be present in the vector, for example, enhancer sequences.

An expression vector is constructed so that the particular coding sequence is located in the vector with the appropriate control and, optionally, regulatory sequences such that the positioning and orientation of the coding sequence with respect to the control sequences allows the coding sequence to be transcribed under the "control" of the control sequences (i.e., RNA polymerase which binds to the DNA molecule at the control sequences transcribes the coding sequence). Modification of the sequences encoding the particular protein of interest may be desirable to achieve this end. For example, in some cases it may be necessary to modify the sequence so that it is attached to the control sequences with the appropriate orientation; i.e., to maintain the reading

frame. The control sequences and other regulatory sequences may be ligated to the coding sequence prior to insertion into a vector. Alternatively, the coding sequence can be cloned directly into an expression vector which already contains the control sequences and an appropriate restriction site.

Preparation of Particulate Nucleic Acid Molecules:

Once obtained and/or constructed, the nucleic acid molecules are prepared for delivery in particulate form. One common method of preparing particulate biopharmaceuticals, such as nucleic acids, is lyophilization (freeze-drying). Lyophilization relates to a technique for removing moisture from a material and involves rapid freezing at a very low temperature, followed by rapid dehydration by sublimation in a high vacuum. This technique typically yields low-density porous particles having an open matrix structure. Such particles are chemically stable, but are rapidly reconstituted (disintegrated and/or brought into solution) when introduced into an aqueous environment.

Another method of providing particulate nucleic acid preparations that can be used with these and other delicate or heat-sensitive biomolecules is spray-drying. Spray-drying relates to the atomization of a solution of one or more solids using a nozzle, spinning disk or other device, followed by evaporation of the solvent from the droplets. More particularly, spray-drying involves combining a highly dispersed liquid preparation (e.g., a solution, slurry, emulsion or the like) with a suitable volume of hot air to produce evaporation and drying of the liquid droplets. Spray-dried pharmaceuticals are generally characterized as homogenous spherical particles that are frequently hollow. Such particles have low density and exhibit a rapid rate of solution.

The low-density particulate solids produced by lyophilization and spray-drying techniques are ideal for redissolution for parenteral administration in solution via syringe or catheters. However, such particles are not useful for delivery from a needleless syringe in a solid form. Accordingly, for purposes of the present method, the preparations are densified to provide particles including nucleic acid molecules that are much better suited for delivery using a needleless syringe (e.g., substantially solid particles having a size of about 50 μm and a density of at least about 0.9 to 1.5 g/cc^3). In particular, the open lattice or hollow shell particles provided by spray-drying or lyophilization can be condensed without heating or shear to provide dense materials that can be milled or otherwise size-reduced to yield pharmaceutical particles having both size and density characteristics suitable for delivery by needleless injection.

The nucleic acids for delivery by the method of the invention are initially prepared in a formulation suitable for spray-drying or lyophilization. Such formulations generally require only a solution in which the nucleic acids will be stable for freezing and lyophilization and, optionally, an excipient for the drying procedure which is acceptable for parenteral delivery. In this regard, suitable excipients may be added to the formulations to provide sufficient mass for an individual dose, enabling measurement of doses by practical processes, e.g., by weight or volume. Typical dosages can be about 0.5 to about 5 mg, preferably about 1 to about 2 mg. Suitable excipients include, but are not limited to, carbohydrates (such as trehalose, glucose, dextrose and sucrose) or polyols (such as mannitol). Amino acids such as glycine and its hydrochloride salt can be used as buffers as well as

phosphate, lactate or citrate buffers, among others. Additionally, any known composition for DNA stabilization will find use in the present formulations. The compositions may optionally include additive agents such as cryoprotectants, antioxidants, or the like. Adjusting compositions to enhance physical and chemical stability of the various particulate nucleic acid formulations provided herein is within the ordinary skill in the art.

One particular approach to stabilization during reprocessing of the nucleic acid formulations entails the use of additives which are combined with the solution prior to freezing for lyophilization to cause the nucleic acids to coil or ball and thus provide the genetic material as a discontinuous phase in the otherwise microscopically homogeneous particles. In such formulations, the bulking agent would be the continuous phase in the dried solid so that any grinding prior to compression, compression densification and regrinding (as described in detail below), and any particle attrition during sizing via sieve or air classification, acceleration and injection, would be less likely to disrupt the long chain nucleic acids. Homogeneity of the particles with respect to nucleic acid content is critical because of the potential for segregation by size during storage or injection.

Condensing the spray-dried or lyophilized powders is conducted by compaction in a suitable press (e.g., a hydraulic press, tableting press or rotary press), wherein the powders are compressed at about 1,000 to 24,000 pounds/square inch (e.g., 0.5 to 12 tons/square inch or 7 to 170 MPa) for a suitable time. Compaction can be carried out under vacuum if desired. The resulting compacted material is then coarsely reground until visually broken up. The particle size is then

reduced to about a 20 to 50 μm average size with an individual particle density of around 0.9 to 1.5 g/cm³. Particle size reduction can be conducted using methods well known in the art including, but not limited to, roller milling, ball milling, hammer or impact milling, attrition milling, sieving, sonicating, or combinations thereof. The compression parameters and particle sizing will, of course, vary depending upon the starting material used, the desired target particle size and density, and like considerations. Particle density can be readily ascertained using known quantification techniques such as helium pycnometry and the like.

Thus, the method can be used to obtain nucleic acid particles having a size ranging from about 10 to about 250 μm, preferably about 10 to about 150 μm, and most preferably about 20 to about 60 μm; and a particle density ranging from about 0.1 to about 25 g/cm³, preferably about 0.8 to about 3.0 g/cm³, and most preferably about 0.9 to about 1.5 g/cm³.

Administration of the Nucleic Acid Molecules:

Following formation, the particulate nucleic acid preparations are delivered to mammalian tissue using a needleless syringe. One needleless syringe for use herein generally is described in commonly assigned International Publication No. WO 94/24263, published 27 October 1994. The syringe comprises an elongate tubular nozzle having a rupturable membrane (or membranes) initially closing the passage through the nozzle and arranged substantially adjacent to the upstream end of the nozzle. The nucleic acid particles to be delivered are disposed adjacent to the rupturable membrane and are delivered using an energizing means which applies a gasecus pressure to the upstream side of the membrane sufficient to burst the membrane and produce a supersonic

gas flow (containing the pharmaceutical particles) through the nozzle for delivery from the downstream end thereof. The particles can thus be delivered from the needleless syringe at delivery velocities of between Mach 1 and Mach 8 which are readily obtainable upon the bursting of the rupturable membrane.

Another needleless syringe configuration generally includes the same elements as described above, except that instead of having the pharmaceutical particles entrained within a supersonic gas flow, the downstream end of the nozzle is provided with a bistable diaphragm which is moveable between a resting "inverted" position (in which the diaphragm presents a concavity on the downstream face to contain the nucleic acid particles) and an active "everted" position (in which the diaphragm is outwardly convex on the downstream face as a result of a supersonic shockwave having been applied to the upstream face of the diaphragm). In this manner, the particles contained within the concavity of the diaphragm are expelled at a supersonic initial velocity from the device for transdermal delivery thereof to a targeted skin or mucosal surface.

Transdermal delivery using the above-described needleless syringe configurations is carried out with particles having an approximate size that generally ranges between 10 and 250 μm. The optimal particle size is usually at least about 10 to 15 μm (the size of a typical cell). Nucleic acid particles larger than about 250 μm can also be delivered from the device, with the upper limitation being the point at which the size of the particles would cause untoward damage to the skin. The actual distance which the delivered particles will penetrate depends upon particle size (e.g., the nominal particle diameter assuming a roughly spherical particle geometry), particle density, the initial velocity at

which the particle impacts the skin surface, and the density and kinematic viscosity of the skin. In this regard, optimal particle densities for use in needleless injection generally range between about 0.1 and 25 g/cm^3, preferably between about 0.9 and 1.5 g/cm^3, and injection velocities generally range between about 200 and 3,000 m/sec.

A particularly unique feature of the needleless syringe is the ability to closely control the depth of penetration of delivered particles, thereby allowing for targeted administration of the nucleic acids to various sites. For example, particle characteristics and/or device operating parameters can be selected to provide penetration depths of less than about 1 mm for intradermal delivery, or approximately 1-2 mm for subcutaneous delivery. One approach entails the selection of particle size, particle density and initial velocity to provide a momentum density (e.g., particle momentum divided by particle frontal area) of between about 2 and 10 kg/sec/m, and more preferably between about 4 and 7 kg/sec/m. Such control over momentum density allows for precisely controlled, tissue-selective delivery of the particulate nucleic acids.

Compositions containing a therapeutically effective amount of the powdered nucleic acid molecules described herein can be delivered to any suitable mammalian tissue via the above-described needleless syringes. For example, the compositions can be delivered to muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland and connective tissues. The nucleic acid molecules are preferably delivered to, and expressed in, terminally differentiated cells; however, the molecules can also be delivered to

non-differentiated, or partially differentiated cells such as stem cells of blood and skin fibroblasts.

The powdered compositions are administered to the subject to be treated in a manner compatible with the dosage formulation, and in an amount that will be prophylactically and/or therapeutically effective. The amount of the composition to be delivered, generally in the range of from 0.5 μg/kg to 100 μg/kg of nucleic acid molecule per dose, depends on the subject to be treated. The exact amount necessary will vary depending on the age and general condition of the individual to be treated, the severity of the condition being treated and the particular nucleotide sequence or sequences selected, the site of administration, as well as other factors. An appropriate effective amount can be readily determined by one of skill in the art.

Thus, a "therapeutically effective amount" of the present powdered nucleic acid molecule compositions will be sufficient to bring about treatment or prevention of disease or condition symptoms, and will fall in a relatively broad range that can be determined through routine trials.

C. Experimental

Below are examples of specific embodiments for carrying out the present invention. The examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperatures, etc.), but some experimental error and deviation should, of course, be allowed for.

Example

The following experiment was conducted to investigate the possibility of using freeze-dried DNA as an alternative to DNA-coated metal particles in the biolistic transfer of genetic material. In particular, powdered DNA plasmids as well as DNA-coated tungsten particles as controls were delivered *ex vivo* to male human fibroblast HT1080 cells using a needleless syringe apparatus as follows.

Clone 123 is a small plasmid of ~11 kb which contains the β-galactosidase marker gene so that transient transformation can be measured with the chromogenic indicator X-Gal. Plasmids were bulked with a carbohydrate excipient, trehalose. Trehalose was selected as the excipient because of its stabilizing properties (Colaco et al. (1992) *Bio/Technology* 10:1009). The trehalose was dissolved in distilled water and filter-sterilized prior to adding the DNA to the solution. Three different solutions of DNA sugar were made up with the proportions shown below in Table 1.

Table 1

Preparation	1	2	3
Clone 123 (2.7μg/μL)	800 μg (296.3 μL)	160 μg (59.3 μL)	80 μg (29.6 μL)
Trehalose (100 mg/15 ml H_2O)	10 mg (1.5 ml)	10 mg (1.5 ml)	10 mg (1.5 ml)
Payload (for 8 μg DNA)	0.1 mg	0.5 mg	1.0 mg

The plasmid/sugar solutions were then freeze-dried (using solid CO_2 and isopropanol to freeze the solution prior to vacuum drying), and the freeze-dried DNA-trehalose solid

milled to form microparticles using an agate mortar and pestle.

As a control, DNA-coated tungsten particles were made by coating tungsten microprojectiles (19.35 x 10^3 kb.m^{-1}) of 1.014 μm median diameter (M-17, GTE/Sylvania, Towanda, PA, USA) with forty micrograms of Clone 123 plasmid DNA, giving five payloads of 8 μg DNA, using a derivative of known methods for coating microparticles. Potter et al. (1984) *Proc. Natl. Acad. Sci. USA* 81:7161, Klein et al. (1987) *Nature* 327:70, and Williams et al. (1991) *Proc. Natl. Acad. Sci. USA* 88:2726.

More particularly, prior to coating, the tungsten particles were sterilized and brought into suspension. A 50 mg sample of 1.048 μm (median diameter) tungsten microprojectiles (M-17, GTE/Sylvania, Towanda, PA, USA) was weighed into a 1.5 cc Eppendorf tube and then sterilized in 100% ethanol (EtOH). In order to disperse the microprojectiles (disrupt particle aggregates), the sterilized solution was sonicated thoroughly by contacting the outside of the Eppendorf tube with the probe of a sonicator. The dispersed tungsten particles were centrifuged and the supernatant removed. The tungsten particles were resuspended in 1 cc sterile distilled water and centrifuged for two cycles, and then stored in 1 cc sterile distilled water until coating.

The plasmid DNA was absorbed to the tungsten microprojectiles by adding 20 μL of the DNA (1 mg/mL) to 40 μL of the suspension of tungsten particles prepared above. The suspension was vortexed to ensure adequate mixing of the reagents. The following reagents were then added, in the order given, with vortexing after each addition: 253 μL $CaCl_2$ (2.5 M); 50 μL spermidine (0.10 M, stored frozen); and 207 μL sterile distilled H_2O. The

final mixture was vortexed for 10 minutes at 4 °C. The DNA-coated tungsten microprojectiles were then centrifuged at 500 G for 5 minutes. After centrifugation, all supernatant was carefully removed and 100 μL of 70% EtOH added. The coated particles were again centrifuged, all supernatant removed, and the final preparation resuspended in 30 μL of 100% EtOH.

The above method resulted in suitable quantities of DNA-coated tungsten particles to allow for 4 to 5 deliveries by the needleless syringe. The above-noted reagent quantities can, of course, be varied to provide different loadings of DNA in accordance with known methods. The volume and molar concentration (M) of the stock solutions used to coat tungsten with DNA are given below in Table 2.

Table 2

Component	Quantity (μL)
Tungsten particles (50 mg/mL)	40
Clone 123 (2.7 μg/μL)	14.8
$CaCl_2$ (2.5 M)	253
Spermidine (0.1 M)	50
distilled H_2O	212.2

For transformation, 6 cm diameter culture dishes were seeded with 5×10^5 male human fibroblast HT1080 cells 24 hours before transfection. Two replicate dishes were prepared for each of the treatments, and two negative control plates were also prepared.

The microparticles and the tungsten-coated particles were then delivered to cells using a needleless syringe as described above. The syringe included a 4.5

mL reservoir chamber with plunger type valve, a helium gas reservoir, a Mach 2 nozzle and 12 μm Mylar sheet hand-punched into 6 mm diameter diaphragms.

In particular, mylar diaphragms were first sterilized by singly layering between pieces of filter paper, stacked one atop the other, wrapped in aluminum foil and sealed completely with autoclave tape to ensure that no water entered the filter paper/diaphragm stack during the autoclave process. This was placed inside a beaker covered with aluminum foil and placed in an autoclave chamber.

Two 12 μm Mylar diaphragms of 6 mm diameter were used in the membrane cassette. One milligram and one-half milligram payloads of the freeze-dried DNA powder were loaded onto the lower membrane in the cassette. This payload was then covered with the other pieces of the cassette and the remaining diaphragm. Only preparations 2 and 3 were used in the experiment because of the difficulty in weighing out small masses accurately. Another five of the cassettes were each loaded with 5 μL of the DNA/tungsten particle suspension. All the above quantities gave a mass of about 8 μg of DNA being delivered in each shot regardless of particle formulation used.

Referring now to Figure 1, a delivery apparatus 2 was assembled which contained a needleless syringe 4 loaded with a cassette as described above. The needleless syringe 4 was arranged on a ring stand 6 using a standard tube clamp 8 to hold the syringe in position relative to a culture dish 10 seeded with the HT1080 cells 12. The distance between the downstream terminus 14 of the needleless syringe 4 and the cells 14 in the culture dish 10 was measured to affix a target distance, generally indicated at d. In order to optimize the parameters for delivery of the freeze-dried DNA, either

the target distance d was varied over a constant delivery pressure, or the delivery pressure was varied over a constant target distance. In particular, the DNA preparations were fired from a target distance ranging from 20 to 60 mm using helium driver gas pressures ranging from 30 to 50 bar.

After transformation, cells were incubated for 2 days, stained, and then transient assays with X-gal were performed to determine transformation efficiencies using previously described methods. Murray, E.J. (ed) (1991) *Methods in Molecular Biology: Gene Transfer and Expression Protocols*, Vol. 7, Humana Press, Clifton, New Jersey. Specifically, transformation efficiency was assessed by counting the number of blue-stained cells. The delivery parameters and transformation results are depicted below in Table 3. Blast effect was rated from 1 point for quite small (diameter of dead cell zone being approximately 5-8 mm) to five points for very large (diameter of cell zone being greater than 30 mm). As can be seen, transformation by the plasmid/trehalose powder preparation of the present invention was on the same order as that observed for the metallic particles.

As shown in Figure 2, optimal transformation results were seen with the particulate plasmid/trehalose preparation when delivered using 30 bar pressure at a target distance of 60 mm. More particularly, Figure 2 provides a direct comparison of the transformation efficiency obtained by delivery of the particulate nucleic acid preparation (both preparations 2 and 3) with historical and contemporary deliveries of DNA-coated tungsten particles. Referring now to Figure 3, data obtained for deliveries at 30 bar are depicted in a graph which presents transformation efficiency as a function of target distance. As can be seen, the optimal target distance for the number 2 and 3 preparations (referred to

as F#2 and F#3, respectively) was not reached; however, transformation efficiency did substantially increase with increased target distances. Further, when deliveries were carried out at the maximum distance tested (60 mm), transformation efficiencies obtained with the particulate DNA formulations (F#2 and F#3) were appreciably better than those observed with the DNA-coated tungsten controls.

In Figs. 2 and 3, the following abbreviations (that are not defined above) apply:

B/P Blue Cell Count per Plate

TCC Tungsten Contemporary Control

THC Tungsten Historical Control

Further, in Fig. 3, the points plotted are as follows:

• F#2

▪ F#3

▲ Tungsten (the point is obscured, at d = 20)

Example 2

The following studies were carried out to assess the ability to deliver a powdered nucleic acid composition to a test subject *in vivo* using the methods of the invention.

Plasmid Vector Construct: The pGREEN-1 vector construct, which contains the Green Fluorescent Protein (GFP) gene under the control of a CMV promoter, was used so that gene expression could be assessed directly by UV microscopy of histological sections from treated tissue samples.

Powdered Nucleic Acid Compositions: A powdered nucleic acid composition was prepared as follows. A mixture was formed by combining pGREEN-1 vector plasmid with trehalose sugar to obtain a 1μg:1mg (w/w) DNA-sugar composition. This composition was lyophilized, compressed, ground, and then sieved, using the techniques described hereinabove. The resulting condensed nucleic acid composition had an average particle size ranging from about 38-75 μm.

Administrations: C57BL/10 mice were treated with 1 mg of the particulate composition via needleless injection. The composition was delivered to a suitably prepared target skin surface, and histological sections were taken from the target site 24 hours after administration. GFP expression was determined directly using UV microscopy. As a result of the administrations, GFP expression was seen in the treated skin tissue, confirming successful *in vivo* delivery of the powdered nucleic acid composition to the target skin, and the subsequent transfection of host cells and expression of the GFP gene therefrom.

In another study, plasmids containing either a human Growth Hormone (hGH) or β-galactosidase (β-Gal) expression cassette were lyophilized with trehalose excipient to form nucleic acid formulations, which were compressed, ground, and then sieved, using the above-described techniques. The resulting condensed nucleic acid compositions had an average particle size ranging from about 38-75 μm.

Female pigs (weighing 20-25 kg) were anesthestised with halothane, and the belly skin was clipped to reveal a suitable target site. The above powdered nucleic acid compositions were individually administered to the prepared target site in 0.1 μg (hGH) or 1 μg (β-Gal) doses via a needleless injection device (delivery pressure of 60 bar). The target sites were biopsied 24 hours after treatment, and histological sections were analyzed for human growth hormone or β-Gal expression. Although no hGH expression was seen within the detection limits of the assay, a moderate degree of β-Gal expression was seen in the treated sites. The lack of detectable hGH expression in this study is due, presumably, to the low loading density of the nucleic acid (0.1 μg) in the composition.

Accordingly, novel methods for DNA delivery have been disclosed. Although preferred embodiments of the subject invention have been described in some detail, it is understood that obvious variations can be made without departing from the spirit and the scope of the invention as defined by the appended claims.

Table 3

Shot	Formulation	Target Distance	Pressure	Blue Cell Count	Blast eff ct
A1	Tungsten	60	30	224	2
A2	Tungsten	60	30	596	2
A3	Tungsten	60	30	575	2
A4	Tungsten	60	30	581	2
A5	Tungsten	60	30	-	-
B1	#3 trehalose	20	30	155	3
B2	#3 trehalose	20	30	227	3
C1	#3 trehalose	40	30	654	2
C2	#3 trehalose	40	30	643	2
D1	#3 trehalose	40	50	394	4
D2	#3 trehalose	40	50	175	5
E1	#3 trehalose	60	30	1416	1
E2	#3 trehalose	60	30	1654	1
F1	#3 trehalose	60	50	408	3
F2	#3 trehalose	60	50	486	3
G1	#2 trehalose	20	30	166	3
G2	#2 trehalose	20	30	180	3
H1	#2 trehalose	20	50	129	4
H2	#2 trehalose	20	50	53	4
J1	#2 trehalose	40	30	347	2
J2	#2 trehalose	40	30	546	2
K1	#2 trehalose	40	50	377	4
K2	#2 trehalose	40	50	198	4
L1	#2 trehalose	60	30	1451	1
L2	#2 trehalose	60	30	1164	1
M1	#2 trehalose	60	50	409	3
M2	#2 trehalose	60	50	336	3

CLAIMS

1. Particles comprising a nucleic acid molecule, not including a metal carrier, for use in therapy.

2. Particles consisting of, or whose mass predominantly comprises, a nucleic acid molecule, for use in therapy.

3. Particles according to claim 1 or claim 2, whose average size is at least as large as that of target cells in skin or mucosal tissue.

4. Particles according to any preceding claim, whose average size is 10 to 250 μm.

5. Particles according to any preceding claim, comprising a carrier.

6. Particles according to claim 5, wherein the carrier comprises trehalose.

7. Particles according to any preceding claim, wherein the nucleic acid molecule comprises a nucleotide sequence encoding an immunogen.

8. Use of particles comprising a nucleic acid molecule, for the manufacture of a medicament not including a metal carrier, for use in therapy by needleless administration to skin or mucosal tissue.

9. Use according to claim 8, wherein the particles are as defined in any of claims 2 to 7.

10. Use according to claim 8 or claim 9, wherein the nucleic acid molecule comprises a gene encoding a protein that is defective or missing from the target cell genome.

11. A needleless syringe comprising, as the active component to be delivered, particles as defined in any of claims 1 to 7.

12. A needleless syringe according to claim 11, adapted to deliver the particles at a momentum density of between 2 and 10 kg/sec/m.

13. A method for delivering particles comprising a nucleic acid molecule to a target cell in skin or mucosal tissue, wherein said particles are administered to the skin or mucosal tissue by needleless syringe and do not include a metal carrier.

14. The method of claim 13, wherein the particles have an average size that is equal to or larger than the size of the target cell.

15. The method of claim 13, wherein the particles have an average size predominantly in the range of about 10 to 250 μm.

16. The method of any of claims 13 to 15, wherein the particles are administered to the skin or mucosal tissue at a momentum density of between 2 and 10 kg/sec/m.

17. The method of any of claims 13 to 16, wherein the particles are delivered to a target cell in epidermal tissue.

18. The method of any of claims 13 to 16, wherein the particles are delivered to a target cell in the stratum basal layer of skin tissue.

19. The method of claims 13 to 18, wherein the particles are comprised of a nucleic acid molecule and a carrier material.

20. The method of claim 19, wherein the carrier material is comprised of trehalose.

21. The method of any of claims 13 to 20, wherein the particles are delivered to the skin or mucosal tissue *in vivo*.

22. The method of any of claims 13 to 20, wherein the particles are delivered to the skin or mucosal tissue *ex vivo*.

23. The method of any of claims 13 to 22, wherein the nucleic acid molecule comprises a gene encoding a protein that is defective or missing from the target cell genome.

24. The method of any one of claims 13 to 22, wherein the nucleic acid molecule comprises a nucleotide sequence encoding an immunogen.

25. A particulate nucleic acid composition suitable for administration to skin or mucosal tissue by needleless syringe, wherein said composition does not include a metal carrier.

ABSTRACT

PARTICLE DELIVERY

Particles comprising a nucleic acid molecule are suitable for needleless injection, to skin or mucosal tissue, without a metal carrier.

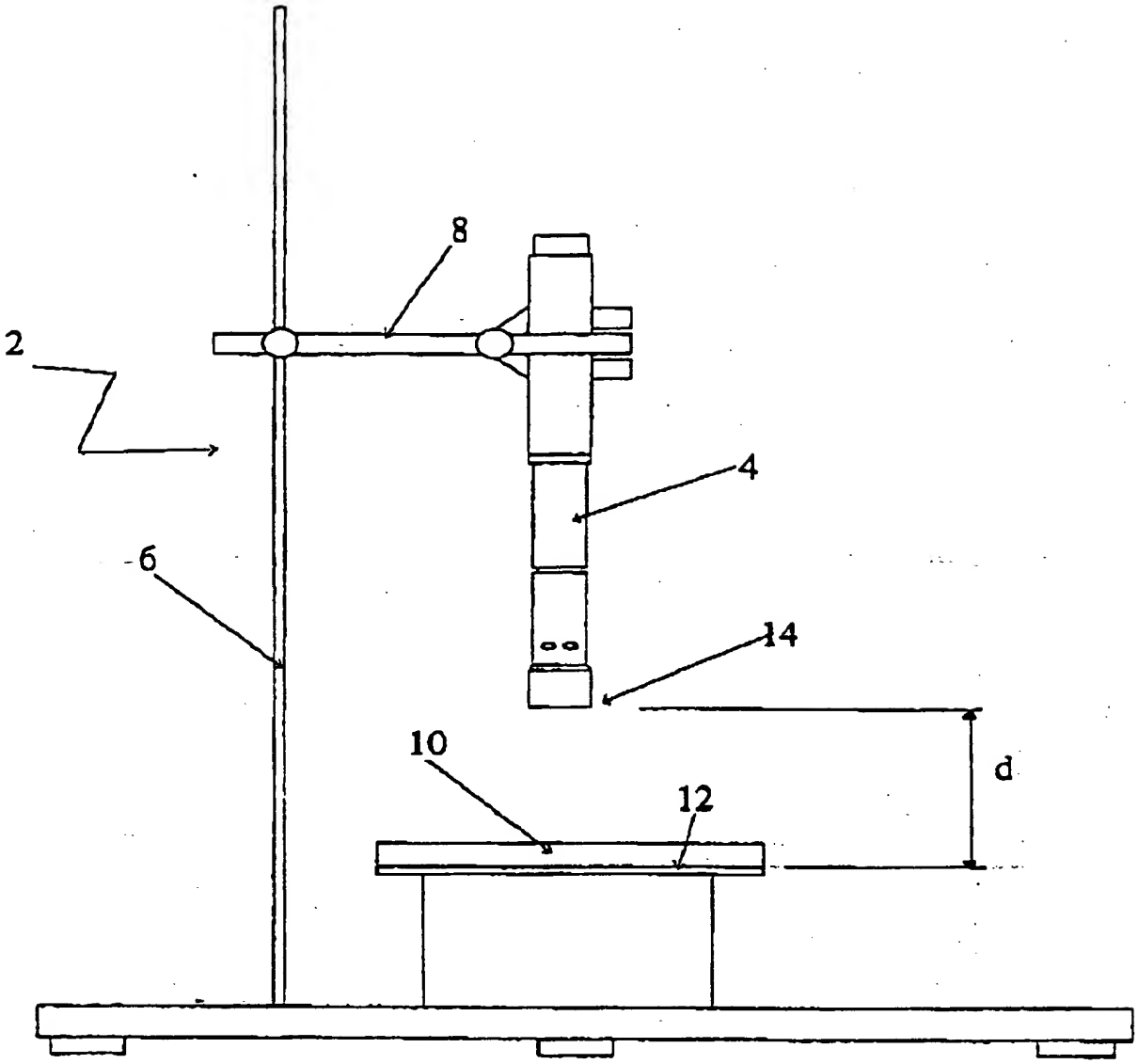

FIG. 1

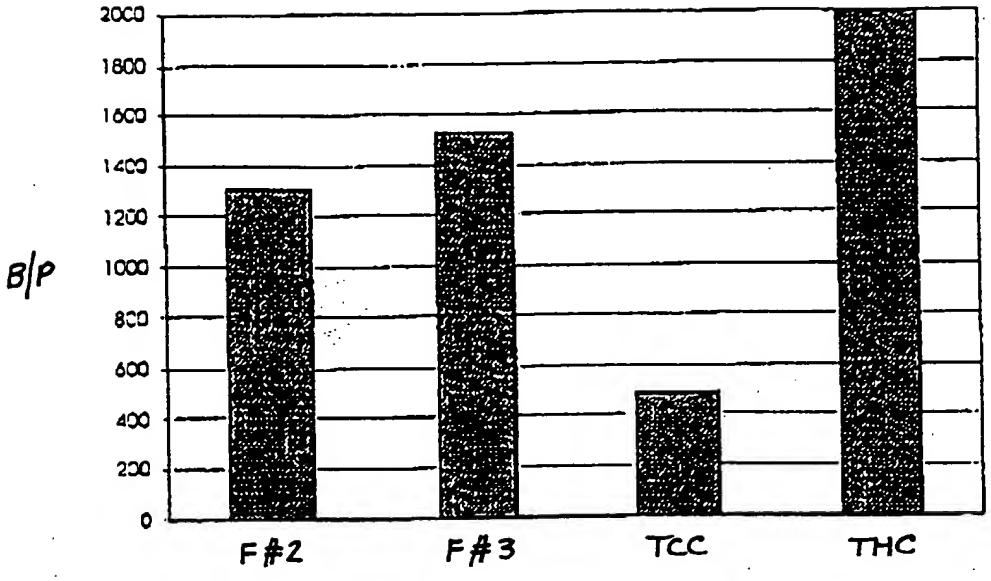
B/P
2000
1800
1600
1400
1200
1000
800
600
400
200
0
F#2
F#3
TCC
THC

FIG. 2

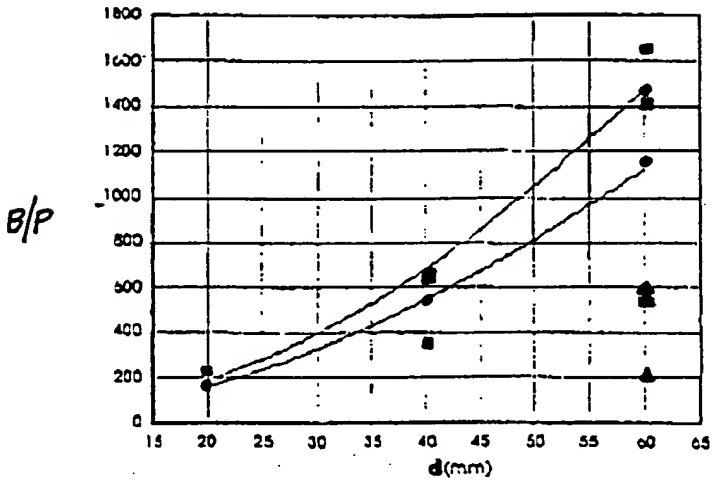
B/P
1800
1600
1400
1200
1000
800
600
400
200
0
15
20
25
30
35
40
45
50
55
60
65
d(mm)

FIG. 3